the abortive transcript pattern seen in lanes b, c, h, and i of the left h panel in Fig. 2 is too complex to be accounted for by presuming one product of each base length. Aligning the transcripts produced in the presence of dGTP only with those produced with dGTP, rUTP, rCTP, and rATP allows us to identify the predominant abortive transcripts in lanes c, d, h, and i of the left panel as poly-dG products. In lane c of the left panel we indicate the major non poly-dG abortives by "4H" and "5H". In lanes b, c, h, and i of the left panel of Fig. 2 we therefore see a pattern similar to that of lane h in Fig. 1. The presence of a mixture of normally extended heterogenous sequence and poly-G abortive transcripts indicates that 'normal' transcript extension is inefficient.

It has been shown that mutations in T7 RNAP that reduce phosphodiester bond formation rates cause poly-rG transcript synthesis even when 4 rNTPs are present (Bonner, et al., 1994). It can be seen that the ratio of poly-G to heterogenous sequence transcripts in lanes b, c, h, i of the left panel of Fig. 2 is greater than in lane h of Fig. 1, indicating a greater deficiency in normal transcript extension when dGTP is substituted for rGTP than when dATP is substituted for dGTP. Note that this occurs even though normal extension of the dGdGdG trimer in lane i of the left panel of Fig. 2 (for example) would involve addition of a *ribo*-AMP while extension of the rGrGrG trimer in lane h of Fig. 1 would involve addition of a *deoxy*-AMP, clearly highlighting the role of transcript structure, as well as substrate structure, in determining the efficiency of transcript extension. These results show that Y639F can initiate and elongate transcripts with dGTP substituted for rGTP but normal extension of the transcripts in the 2-8 base

range is impaired leading to a large increase in the proportion of poly-dG and short transcripts synthesized. Addition of rGMP to serve as the initiating nucleotide and the use of a supercoiled template both enhance the ability of the mutant to extend the short transcripts during the initial stages of transcription.

Barriers to initiation and extension of the initial transcript with dNTPs: Fig. 2 reveals that Y639F can efficiently transcribe with dGTP the initial G segment of a promoter that initiates "GGGA" but is severely blocked in extending the dG trimer with the A. This implies that the sequence of the initially transcribed region may influence the efficiency with which Y639F can extend the transcript when using dNTPs. Fig. 2 also shows that supercoiling, which presumably facilitates unwinding of the template, enhances the activity of Y639F when using dNTPs. To evaluate the effects of sequence and single-strandedness in the initially transcribed region on the activity of Y639F when using dNTPs, we examined transcription from a set of synthetic promoters which differed in the sequence of their initially transcribed regions and in being fully double-stranded or single-stranded in their initially transcribed regions (Fig. 3).

Fig. 3 shows the effects of single-strandedness and sequence in the initially transcribed region on the activity of Y639F in reactions with 4 rNTPs or 4 dNTPs. Poly-rG transcripts of various sizes are indicated in lane a. Reactions contained the indicated NTPs and polymerases. Polymerase and promoter concentrations were 10^{-6} M and 10^{-5} M, respectively. NTP concentrations and electrophoresis as in Fig. 1. Indicated in some of the lanes are the sequences of different transcripts as deduced from alignment with

poly-rG or poly-dG ladders synthesized in the presence of rGTP or dGTP only. The synthetic promoter templates used are double-stranded and have the sequence--CGAAATTAATACGACTCACTATA (SEQ ID NO:2)--in their -23 to -1 regions. The promoters differ in their initially transcribed regions as follows: b-e, t, u: GGACT; f-j, v, w: GAGACCGG; a, j-m, x, y: GGGAGACC; n, o, z: GGAAAATT; p-s: GGGGGGGGGGGACT (SEQ ID NO:3). The promoters also differ in being double-stranded (b, d, f, h, j, l, p, r) or single-stranded (other lanes) in their transcribed regions.

For most of the promoters tested, transcription with rNTPs was not markedly affected by having the initially transcribed region be single-stranded. However, when transcribing with 4 dNTPs, Y639F was more active on the partially single-stranded promoters. For example, in lane e (partially single-stranded promoter) of Fig. 3 transcription products are both more abundant and extend to greater lengths than in lane d, where the promoter is fully double-stranded. A similar comparison may be made between lanes m and l or s and r. Regarding sequence we found that a promoter which initiated with 3 G's was superior to a promoter which initiated with two, which was in turn superior to a promoter which initiated with just one G. Thus Y639F activity when using dNTPs was greatest on a promoter which initiates "GGGAGACC" (lanes l and m). The initially transcribed region of this promoter corresponds to the consensus sequence for T7 promoters in the +1 to +6 segment. This was the only promoter which, when fully double stranded, gave rise to high levels of transcript synthesis with Y639F in reactions containing only dNTPs (lane l).